Supplementary Information

Biocompatible hydrogel coating on single living cells through visible light-induced polymerization

Young Jae Jung[a,b,†], Hyunwoo Choi[b,†], Insung S. Choi*[,b] and Jungkyu K. Lee,*[,a]

[a]*Department of Chemistry and Green-Nano Materials Research Center, Kyungpook National University, Daegu 41566, South Korea.* [†]These authors contributed equally.

[b]*Department of Chemistry, KAIST, Daejeon 34141, South Korea*.

*To whom correspondence should be addressed.

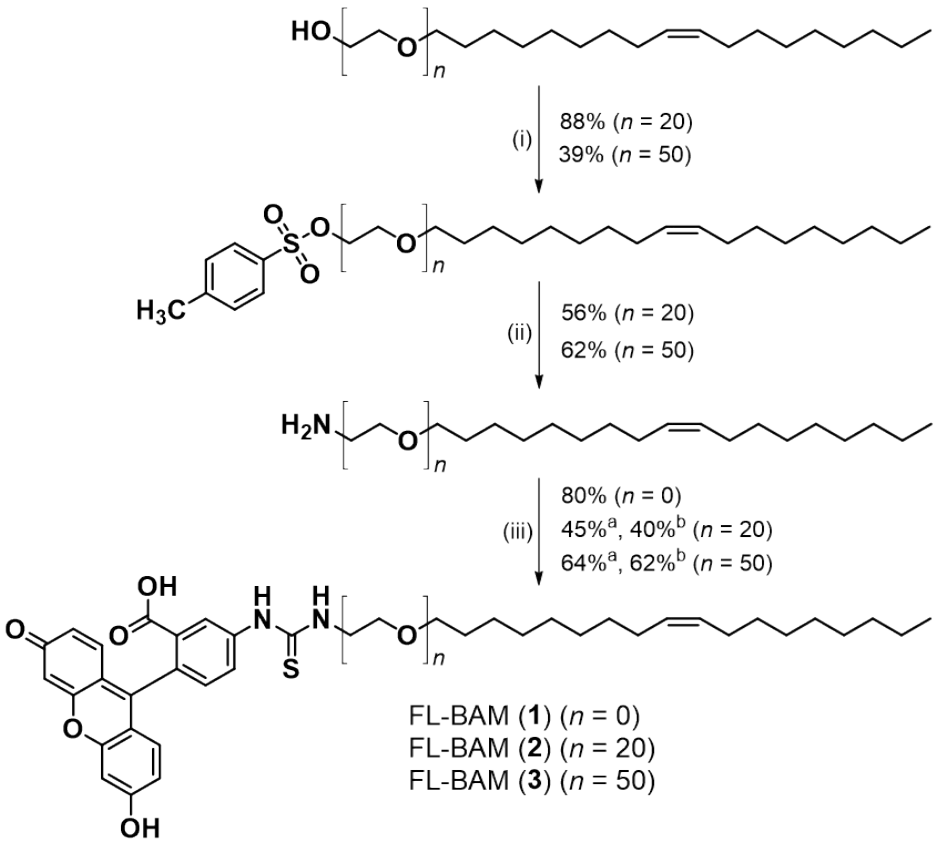
Email: jkl@knu.ac.kr and ischoi@kaist.ac.kr

Tel: +82-53-950-5339

Fax: +82-53-950-6330

1. Materials. poly(ethylene glycol) monooleyl ether (n = ~20 or 50, TCI), *p*-toluenesulfonyl chloride (tosyl chloride, 99%, TCI), fluorescein isothiocyanate (FITC, 90%, Thermo Fisher Scientific), ammonium hydroxide (Junsei), oleylamine (70%, TCI), tetrahydrofuran (anhydrous, 99.7%, Alfa Aesar), potassium carbonate (99.5%, Junsei), sodium sulfate (99%, Junsei), triethylamine (anhydrous, 99.5%, Sigma-Aldrich), NaOH (96%, Junsei), dimethyl sulfoxide (DMSO, anhydrous, 99.9%, Alfa Aesar), dimethyl sulfoxide (DMSO, Sigma-Aldrich), dichloromethane (anhydrous, 99.8%, Sigma-Aldrich), methanol (Duksan Pure Chemicals Co.), hexanes (Duksan Pure Chemicals Co.), ethyl acetate (Duksan Pure Chemicals Co.), membrane tubing (1.0 kDa molecular weight cut-off, Spectrum Labs), Dulbecco's modified Eagle's minimal essential medium (DMEM, with 4500 mg/L D-glucose, L-glutamine, 100 mg/L sodium pyruvate, and sodium bicarbonate, Welgene), Dulbecco's Modified Eagle's Medium (DMEM (w/o phenol red), with 4500 mg/L D-glucose, L-glutamine, 110 mg/L sodium pyruvate ,without phenol red), trypan blue (0.4% solution, Thermo Fisher Scientific), fetal bovine serum (FBS, Welgene), penicillin-streptomycin (5000 U/mL of penicillin and 5000 μg/mL of streptomycin, Welgene), Live/Dead® viability/cytotoxicity kit (Life Technologies), trypsin-ethylenediaminetetraacetic acid solution (0.05% trypsin, 0.53 mM EDTA·4Na in Hank's balanced salt solution, Welgene), glutaraldehyde solution (70% in H_2O, Sigma-Aldrich), phosphate-buffered saline (PBS) solution (10 mM, pH 7.4, Welgene), phosphate-buffered saline (PBS) solution (10×, pH 7.2, Welgene), poly(ethylene glycol) diacrylate (PEGDA, average M_n = 4,000 g/mol, Sigma-Aldrich), triethanolamine (TEOA, Sigma-Aldrich), *N*-vinylpyrrolidone (VP, Sigma-Aldrich), visible LED (wavelength: 530 ± 30 nm, 40.8 mW/cm^2, 110 kLux, Advanced Illumination), and deionized water (DI water, 18.3 MΩ·cm, Milli-Q Direct 8 (Millipore)) were purchased and used without further purification.

2. Experimental procedures and details.



	FL-BAM **(1)**	FL-BAM **(2)**	FL-BAM **(3)**
n	0	20[a], 22[b]	50[a], 36[b]
M.W. (g/mol)	656.3[a,b]	1537.9250[a] 1626.0301[b]	2859.5018[a] 2241.2457[b]
% $_{FL}$[a]	100	70	85.8

Fig. S1 Synthetic procedure for fluorescein-terminated BAMs (FL-BAMs) and relative abundance of FL within FL-BAMs: (i) 4-toluenesulfonyl chloride (1.5 equiv.), dropwise, 0 °C → rt, THF, 12 h; (ii) 28 % ammonia (aq) (xs), rt, 5 d; (iii) fluorescein isothiocyanate (1.2 equiv.), triethyl amine (2.0 equiv.), dark, 12 h, THF, rt, for *n* = 0 and DMSO, reflux for *n* = 20 and 50. [a] Calculated value based on ^{1}H-NMR. [b] Calculated value based on mass spectroscopy.

Synthesis of poly(ethylene glycol) monooleyl ether tosylate (Ts-BAM, *n* = 20). Poly(ethylene glycol) monooleyl ether (5.0 g, 4.3 mmol, 1 equiv.) was dissolved in 10 mL of tetrahydrofuran (THF) at 0 °C. An aqueous solution of NaOH (6.25 M, 2.5 mL) was added dropwise to the stirred solution of poly(ethylene glycol) monooleyl ether for 30 min. Subsequently, tosyl chloride (1.26 g, 6.6 mmol, 1.5 equiv.) dissolved in 20 mL of THF was slowly added to the reaction mixture. The reaction mixture was then allowed to room temperature and stirred for 4 h. The solvent was removed by rotary evaporation under reduced pressure. The resulting residue was then dissolved in dichloromethane and washed with water. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated again under reduced pressure. Finally, the crude product was recrystallized from diethyl ether to yield a white solid (4.9 g, 3.8 mmol, 88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.79 (d, 2H, *J* = 8.2 Hz), 7.33 (d, 2H, *J* = 7.9 Hz), 5.33 (t, 2H, *J* = 5.5 Hz and 5.3 Hz), 4.15 (t, 2H, *J* = 4.9 Hz and 4.7 Hz), 3.64 (m, 77H), 3.43 (t, 2H, *J* = 6.7 Hz and 6.9 Hz), 2.45 (s, 3H), 1.96 (q, 4H, *J* = 6.6 Hz, 5.9 Hz and 6.6 Hz), 1.57 (m, 2H), 1.25 (m, 23H), 0.86 (t, 3H, *J* = 7.1 Hz and 6.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.78, 133.04, 129.93, 129.84, 129.83, 127.99, 71.55, 70.57 (PEG), 69.29, 68.69, 31.90, 29.31 (oleyl), 27.21, 26.10, 22.68, 21.65, 14.13.

Synthesis of poly(ethylene glycol) monooleyl ether tosylate (Ts-BAM, *n* = 50). The same procedure was followed as described above (411.6 mg, 0.15 mmol, 39 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77 (d, 2H, *J* = 8.4 Hz), 7.32 (d, 2H, *J* = 8.1 Hz), 5.35 (t, 2H, *J* = 5.3 Hz and 5.5 Hz), 4.17 (t, 2H, *J* = 4.8 Hz and 4.9 Hz), 3.64 (m, 201H), 3.41 (t, 2H, *J* = 6.9 Hz and 6.8 Hz), 2.43 (s, 3H), 1.97 (q, 4H, *J* = 6.7 Hz, 5.8 Hz and 6.6 Hz), 1.54 (m, 2H), 1.24 (m, 23H), 0.86 (t, 3H, *J* = 7.0 Hz and 6.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.78, 133.07, 129.94, 129.85, 129.83, 127.99, 71.55, 70.59 (PEG), 31.90, 29.32 (oleyl), 27.22, 26.11, 22.68, 21.66, 14.12.

Synthesis of amine-terminated poly(ethylene glycol) monooleyl ether (Aminated BAM, *n* = 20). Ts-BAM (1.0 g, 0.77 mmol) was reacted with an excess of ammonium hydroxide (28 wt% aqueous solution, 2.5 mL, 37 mmol) at room temperature for 5 days. The reaction mixture was then extracted with dichloromethane to isolate the product. The organic layer was dried over anhydrous Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The crude product was recrystallized from diethyl ether to yield aminated BAM as a solid (0.49 g, 0.43 mmol, 56%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.28 (t, 2H, *J* = 5.1 Hz and, 5.2 Hz), 3.63 (m, 90H), 3.40 (t, 2H, *J* = 6.7 Hz and 6.9 Hz), 2.84 (t, 2H, *J* = 5.3 Hz and 5.2 Hz), 1.99 (m, 4H), 1.84 (s, 2H), 1.55 (m, 2H), 1.24 (m, 23H), 0.85 (t, 3H, *J* = 7.2 Hz and 6.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 129.90, 129.82, 73.42, 71.52, 70.55 (PEG), 41.79, 31.86, 29.74 (oleyl), 27.18, 26.07, 22.66, 14.11.

Synthesis of amine-terminated poly(ethylene glycol) monooleyl ether (aminated BAM, *n* = 50). The same procedure was followed as described above (411.6 mg, 0.2 mmol, 38 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.35 (t, 2H, *J* = 4.9 Hz and 5.0 Hz), 3.66 (m, 204H), 3.45 (t, 2H, *J* = 6.7 Hz and 6.9 Hz), 2.88 (t, 2H, *J* = 5.1 Hz and 5.2 Hz), 2.00 (m, 6H), 1.58 (m, 2H), 1.28 (m, 23H), 0.89 (t, 3H, *J* = 7.0 Hz and 7.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 129.93, 129.85, 73.32, 71.55, 70.57 (PEG), 41.77, 31.90, 29.77 (oleyl), 27.21, 26.09, 22.68, 14.12.

Synthesis of oleyl fluorescein (FL-BAM 1). Fluorescein isothiocyanate (25.8 mg, 60 μmol, 1 equiv.) was dissolved in anhydrous THF (3 mL) under an argon atmosphere. Oleyl amine (52 mg, 150 μmol, 2.5 equiv.) and triethylamine (24 mg, 240 μmol, 4 equiv.) were then added sequentially to the reaction mixture. The reaction was stirred under an argon atmosphere for 12 h. The solvent was removed by rotary evaporation under reduced pressure. The crude product was purified by silica gel column chromatography using a mixture of dichloromethane, methanol, and acetic acid (20:1:1, v/v/v) as the eluent. This yielded the desired oleyl fluorescein

(FL-BAM 1) as an orange solid (31.2 mg, 47.5 μmol, 80%). ^{1}H NMR (500 MHz, DMSO-*d*): δ 10.17 (broad s, 2H), 9.89 (s, 1H), 8.25 (s, 1H), 8.09 (s, 1H), 7.72(d, 2H, *J* = 6.1 Hz), 7.16 (d, 1H, *J* = 8.3 Hz), 6.68 (d, 2H, *J* = 2.1 Hz), 6.60 (d, 2H, J = 8.7 Hz), 6.55 (dd, 2H, *J* = 8.7 Hz and 2.3 Hz), 5.32 (t, 2H, *J* = 5.8 Hz and 5.7 Hz), 3.49 (broad s, 2H), 1.98 (m, 4H), 1.57 (m, 2H), 1.23 (m, 23H), 0.84 (t, 3H, *J* = 7.0 Hz and 6.7 Hz); ^{13}C NMR (125 MHz, DMSO-*d*): δ 180.80, 169.02, 160.06, 152.39, 141.89, 130.55, 130.52, 130.10, 130.09, 129.48, 124.49, 113.10, 110.25, 102.71, 44.28, 31.75, 29.05 (m, oleyl), 27.06, 27.03, 26.90, 22.56, 14.41; HR-MS: *m/z* = 657.3342 $[M+H]^+$ (cal. 656.33 $[M]^+$).

Synthesis of fluorescein-terminated poly(ethylene glycol) monooleyl ether (FL-BAM 2). Fluorescein isothiocyanate (FITC, 20 mg, 52 μmol, 1.2 equiv.) and aminated BAM (50 mg, 43.5 μmol, 1 equiv.) were dissolved in 1 mL of anhydrous dimethyl sulfoxide under an argon atmosphere. Triethylamine (9 mg, 87 μmol, 2 equiv.) was added, and the reaction mixture was stirred at 60 °C for 12 h. After reaction, the crude product was cooled and purified by membrane dialysis using a membrane tube (1 kDa molecular weight cut-off) against methanol. The solvent was replaced four times. After dialysis, the product was concentrated under reduced pressure to yield an orange oil (30.3 mg, 45%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.00 (broad s , 0.49H), 8.17 (broad s, 0.72H), 7.99 (broad s, 0.62H), 7.56 (broad s, 0.59H), 7.08 (d, 0.74H, *J* = 8.0 Hz), 6.77 (s, 1.39H), 6.67 (d, 1.42H, *J* = 8.6 Hz), 6.57 (d, 1.40H, *J* = 8.0 Hz), 5.34 (t, 1.52H, *J* = 3.9 Hz and 4.7 Hz), 3.64 (m, 92H), 3.45 (t, 2H, *J* = 6.9 Hz and 6.8 Hz), 2.00 (m, 4H), 1.57 (m, 2H), 1.27 (m, 22H), 0.86 (t, 3H, *J* = 7.2 Hz and 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 129.93, 129.85, 71.56, 70.55 (PEG), 31.90, 29.77 (oleyl), 27.21, 26.08, 22.69, 14.13; MS (MALDI-ToF): *m/z* = 1648.6990 $[M+Na^+]^+$ (cal. 1626.0301 $[M_{(n=22)}]$).

Synthesis of fluorescein-terminated poly(ethylene glycol) monooleyl ether (FL-BAM 3). The same procedure was followed as described above (80.2 mg, 28 μmol, 64 %). ^{1}H NMR (500

MHz, $CDCl_3$): δ 9.06 (broad s , 0.64H), 8.16 (s, 0.78H), 8.00 (d, 0.79H, *J* = 7.9 Hz), 7.61 (broad s, 0.65H), 7.08 (d, 0.90H, *J* = 8.2 Hz), 6.75 (s, 1.63H), 6.67 (d, 1.72H, *J* = 8.7 Hz), 6.56 (dd, 1.80H, *J* = 8.5 Hz, 2.0 Hz and 1.8 Hz), 5.33 (t, 1.59H, *J* = 5.3 Hz and 5.5 Hz), 3.64 (m, 215H), 3.44 (t, 2H, *J* = 6.8 Hz and 6.7 Hz), 2.00 (m, 4H), 1.57 (m, 2H), 1.27 (m, 22H), 0.86(t, 3H, *J* = 7.2 Hz and 6.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 129.94, 129.85, 71.56, 70.56 (PEG), 31.90, 29.77 (oleyl), 27.21, 26.09, 22.69, 14.13; MS (MALDI-ToF): *m/z* = 2272.057 $[M+Na^+]^+$ (cal. 2241.2457 $[M_{(n = 36)}]$).

Cell culture. NIH 3T3 fibroblast cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. Cells were maintained at 37 ℃ under humidified atmosphere with 5% CO_2 (g). When the cells reached approximately 80% confluency, they were passaged. The culture flask was rinsed twice with phosphate-buffered saline (PBS) (aq). Upon the addition of trypsin (2 mL), the flask was incubated at 37 ℃ for 5 min to detach the cells. After the detachment, 3 mL of DMEM was added. The cell suspension was collected by centrifugation. Finally, the cells were washed twice with PBS (aq).

Viability test. Cell viability on the substrates was assessed using Live/Dead® Viablility/Cytotoxicity Kit for mammalian cells. The calcein AM (0.5 μL) and ethidium homodimer-1 (2 μL) were dissolved in 1 mL PBS. The resulting solution was added to each substrate after washing with PBS, and the cells were incubated for 20 min. After incubation, the fluorescence of the stained cells was analyzed using CLSM.

Cell Stability test. Bare cells and hydrogel-coated cells were suspended in 1 mL of phenol red free DMEM. Each sample was placed in a falcon tube and left it under ambient conditions for analysis using CLSM.

Immobilization of FL-BAM on the cell surfaces. A stock solution of 10 mM FL-BAMs (10

mM in DMSO) was diluted in phenol red-free DMEM to various concentrations (1, 5, and 10 uM), ensuring the final DMSO concentration was less than 1%. The cells (10^6 cells/mL) were incubated with the diluted solutions at 37 ℃ for different time points (2, 6, and 10 min). After incubation, the cells were centrifuged at 300 × g for 3 minutes at 25°C, first with phenol red-free DMEM and then with PBS (aq). The cells were then resuspended in 100 μL of PBS (aq) for CLSM analysis.

Preparation of fluorescent nanoparticles. To minimize nonspecific binding of fluorescent nanoparticles (diameter: 20 nm) to cells, a 1% (w/v) solution of the nanoparticles was incubated with 1% (w/v) bovine serum albumin (BSA) in PBS (aq) for 2 h. Following the incubation, the mixture was centrifuged using an ultracentrifuge filter (MWCO: 300 kDa) device at 6,000 rpm for 10 min to remove unbound BSA. The concentrated nanoparticle solution was further washed twice with PBS (aq) using the ultracentrifuge device. The concentration of fluorescent nanoparticles in the final solution was adjusted to 1% (w/v), and 2 mM sodium azide was added. The modified fluorescent nanoparticles were then stored at 4 ℃ for future use.

Visible light-induced polymerization from FL-BAM anchored cells. The cells treated with FL-BAM were resuspended in a phenol red-free DMEM solution (total volume: 310 μL) containing PEGDA 4000 (30 mM), TEOA (5 mM), VP (35 mM), and 10 μL of the prepared fluorescent nanoparticles solution. The cell suspension was then spread onto a sterilized slide glass (2.5 cm × 2.5 cm). The slide was irradiated with the visible LED for 10 min to initiate photopolymerization. After irradiation, the reaction mixture was carefully collected using a pipette. The resulting cells were then washed twice with phenol red-free DMEM. The hydrogel-coated cells were resuspended into 100 μL PBS (aq) for analysis using CLSM.

Sample preparation of SEM. The encapsulated cells were fixed in 1.4 mL of a solution containing 40 μL of 70% glutaraldehyde, 140 μL of 10× PBS (aq), and 1220 μL of DI water at

4 ℃ for overnight. After fixation, the cells were washed three times with PBS (aq) and DI water and then spread on a gold plate for drying.

3. Characterizations.

Field-emission scanning electron microscopy (FE-SEM). The SEM images obtained with a FEI Inspect F50 microscope with an accelerating voltage of 5.0 kV, after sputter-coating with platinum.

Mass and nuclear magnetic resonance (NMR) spectroscopy. Mass spectrometry (HRMS) analysis was performed on a XEVO G2-XS QToF mass spectrometer (Waters^{TM} Corp.) for high-resolution and MALDI-ToF/ToF (Aautoflex maX, Bruker). ^{1}H and ^{13}C NMR spectra were obtained by Bruker Advance 500 NMR spectrometer (Bruker).

4. Supporting figures

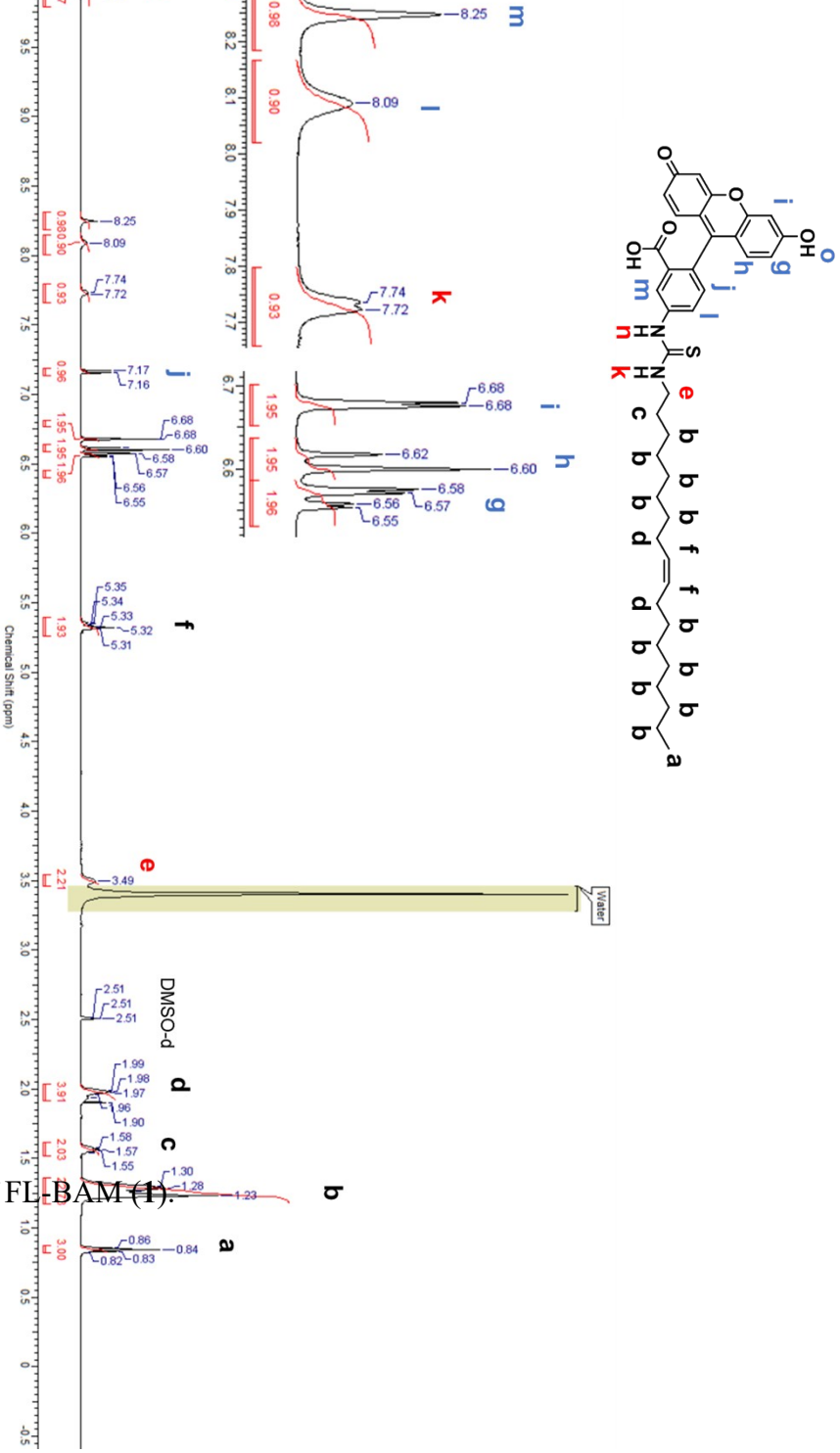


Fig. S2 ^{1}H-NMR spectrum of FL-BAM (1).

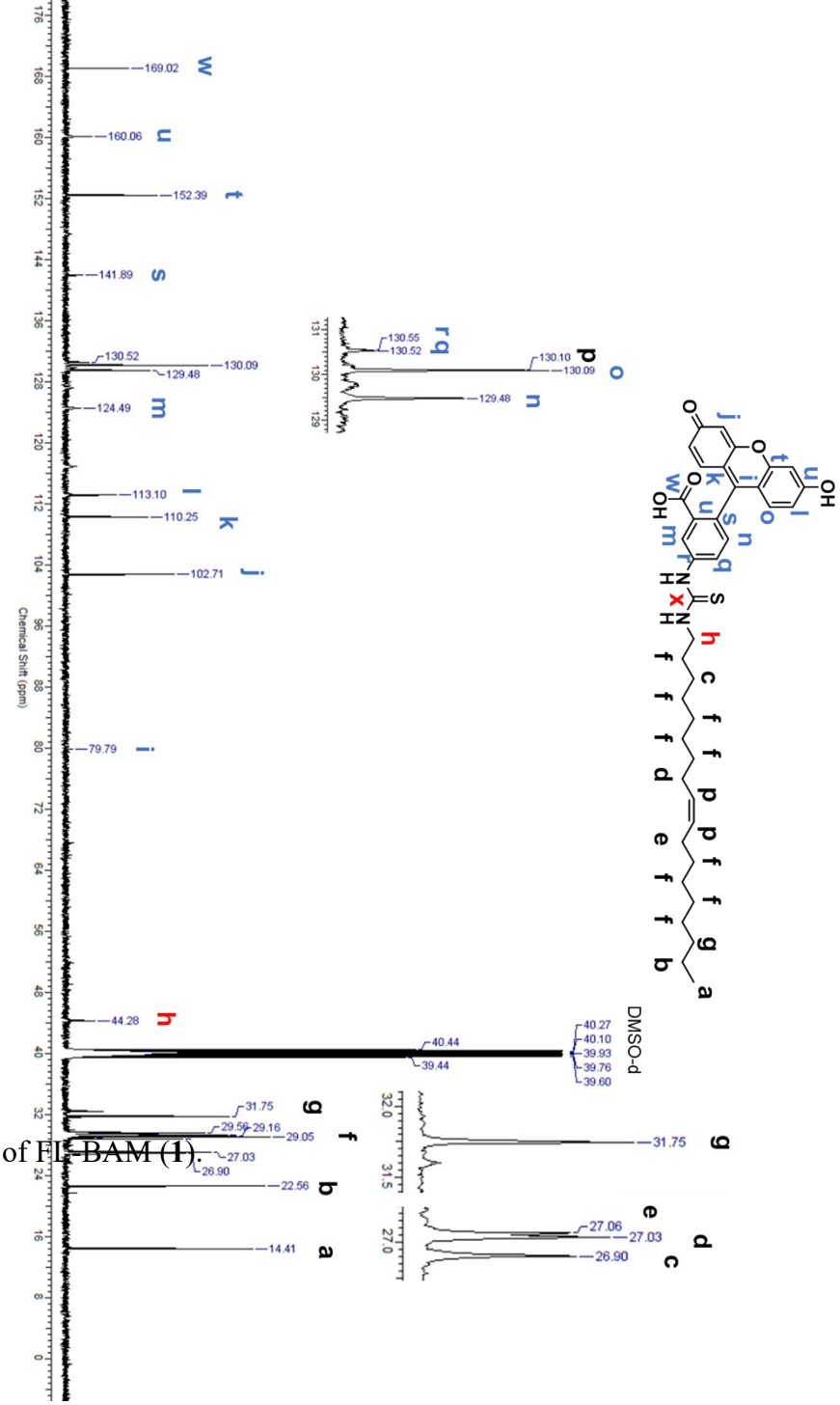


Fig. S3 ^{13}C-NMR spectrum of FL-BAM (1).

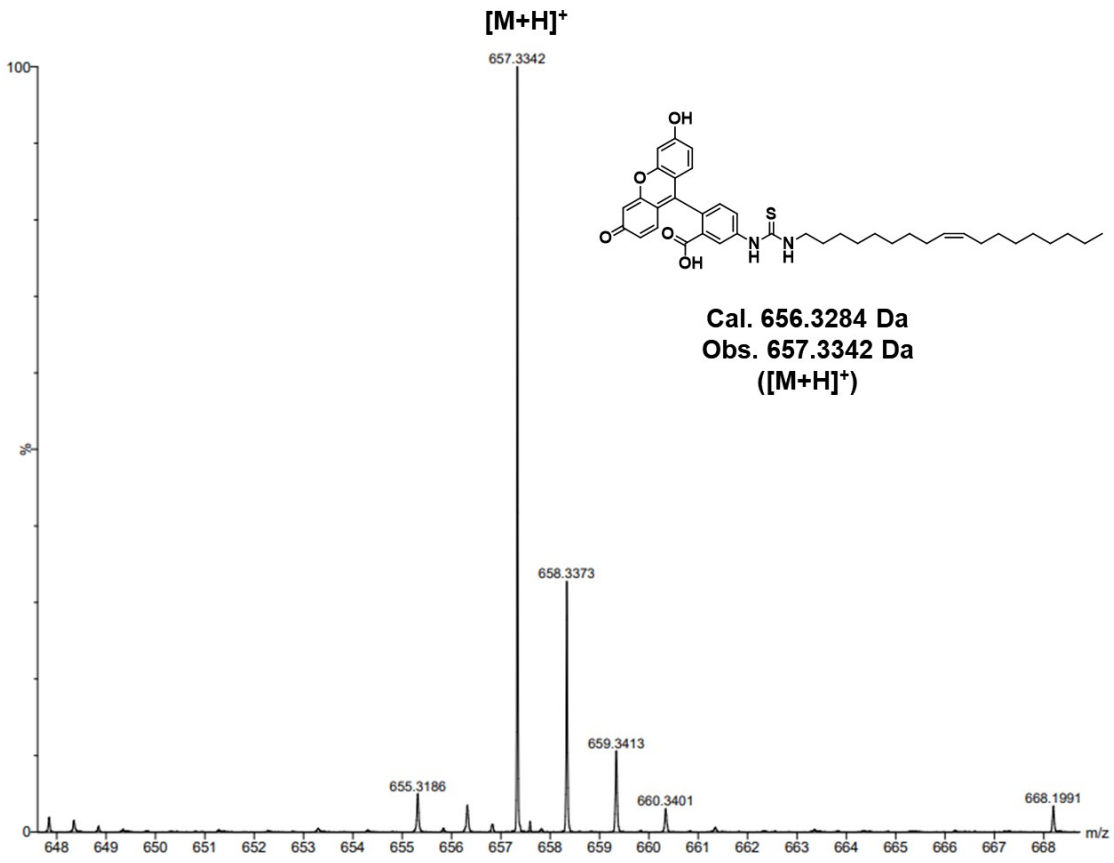


Fig. S4 High-resolution mass spectrum of FL-BAM (**1**).

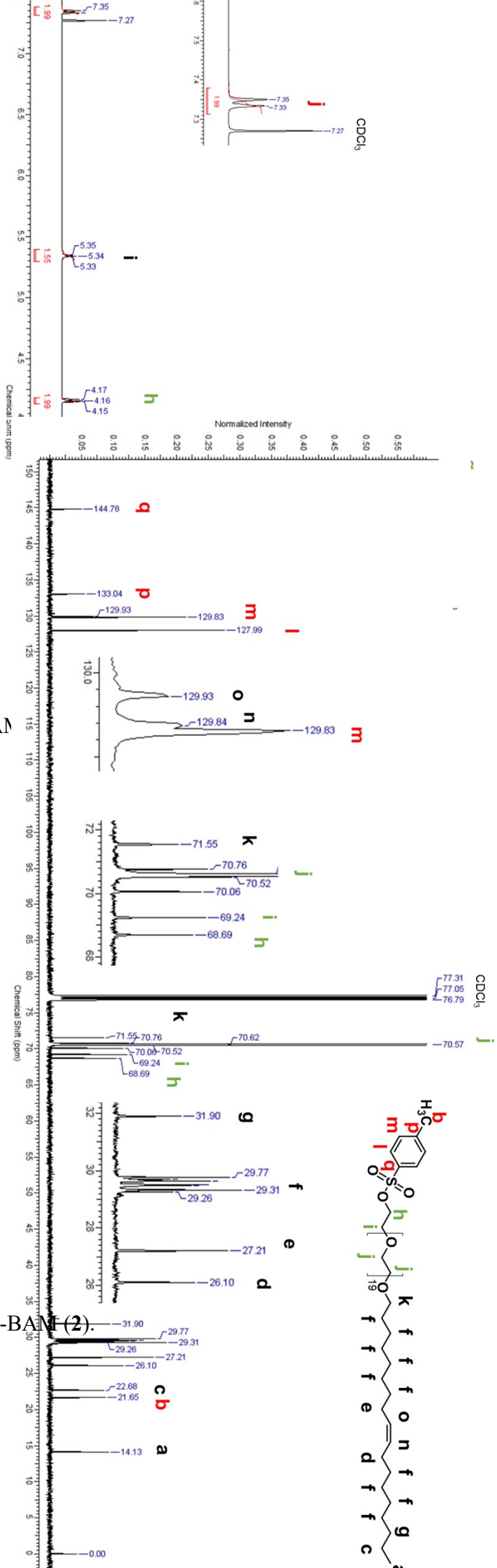


Fig. S5 [1]H-NMR spectrum of Ts-BAM

Figure S6. [13]C-NMR spectrum of Ts-BAM (**2**).

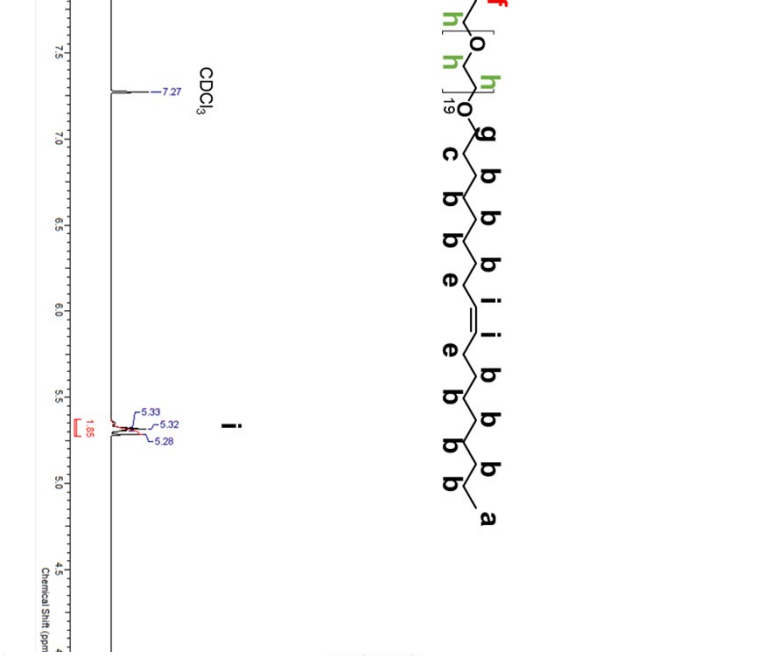


Figure S7. [1]H-NMR spectrum of ar

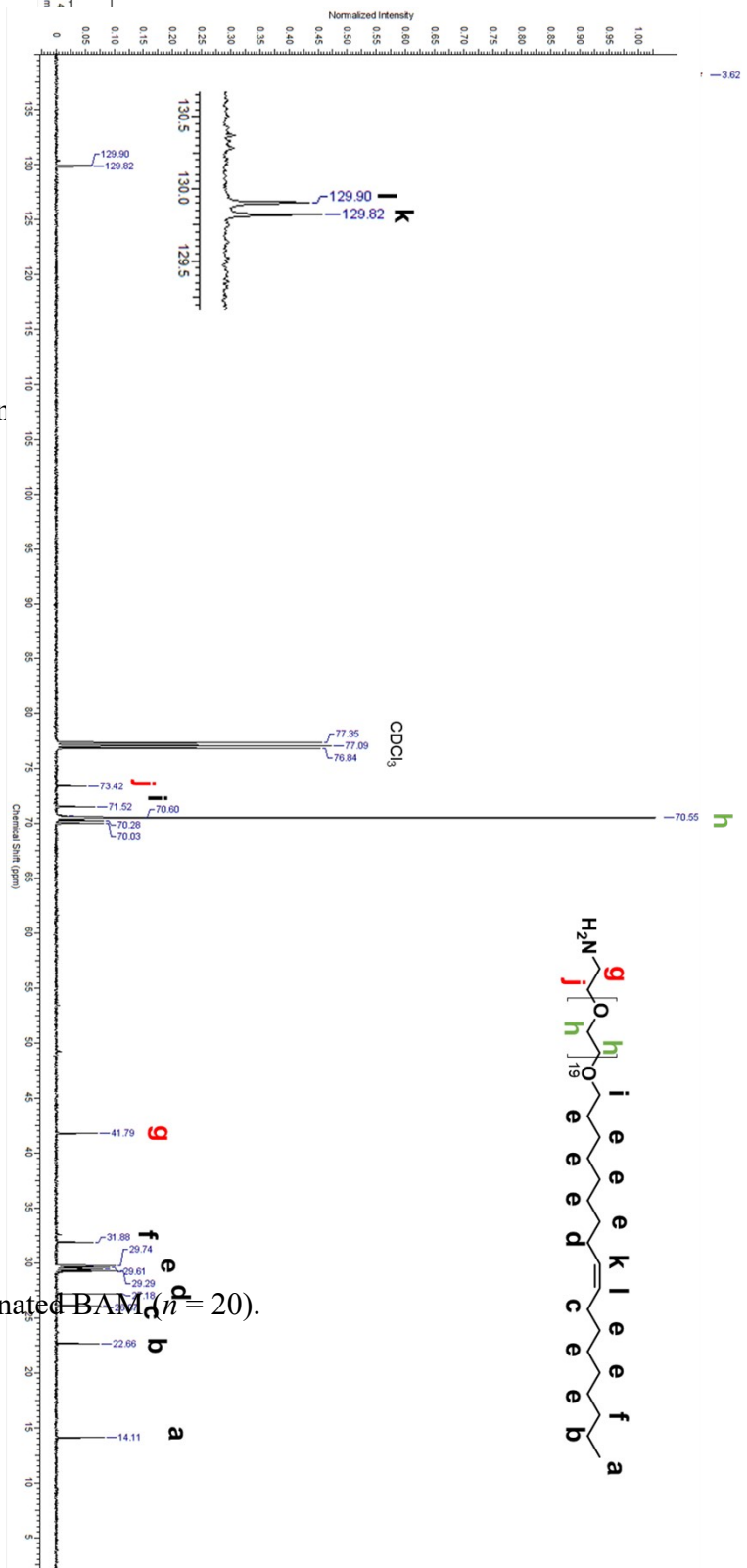


Fig. S8 [13]C-NMR spectrum of aminated BAM (n = 20).

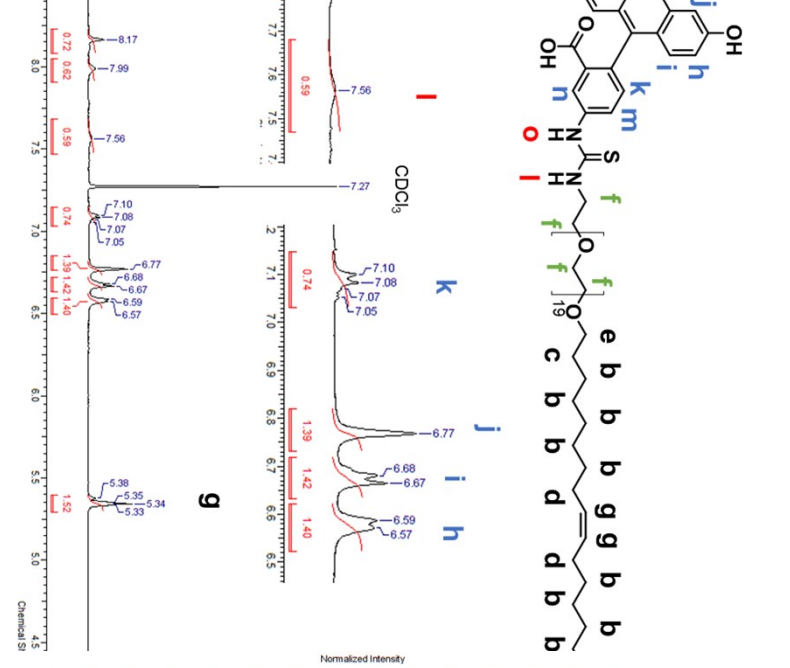


Fig. S9 ^{1}H-NMR spectrum of FL-B

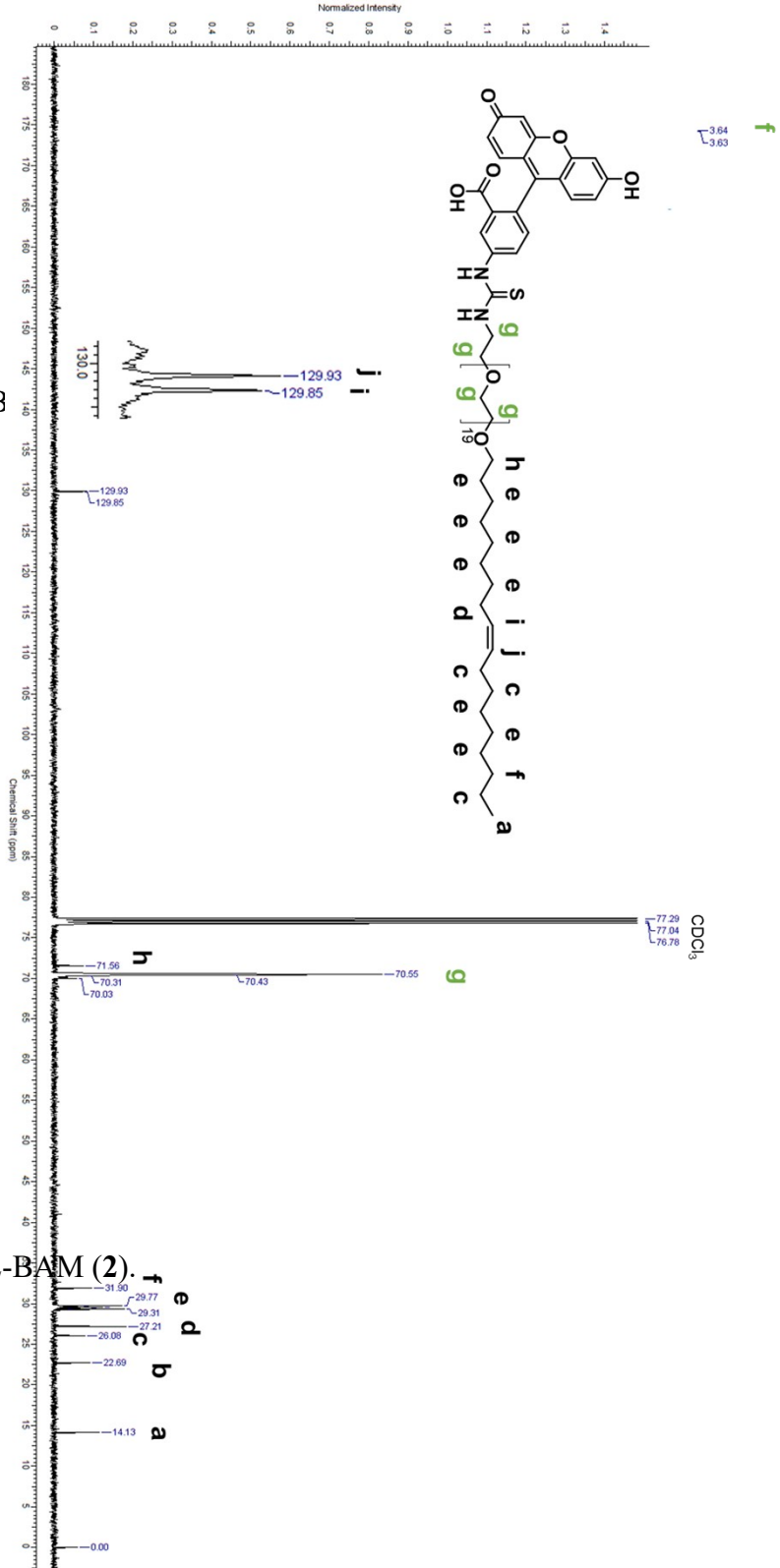


Fig. S10 ^{13}C-NMR spectrum of FL-BAM (**2**).

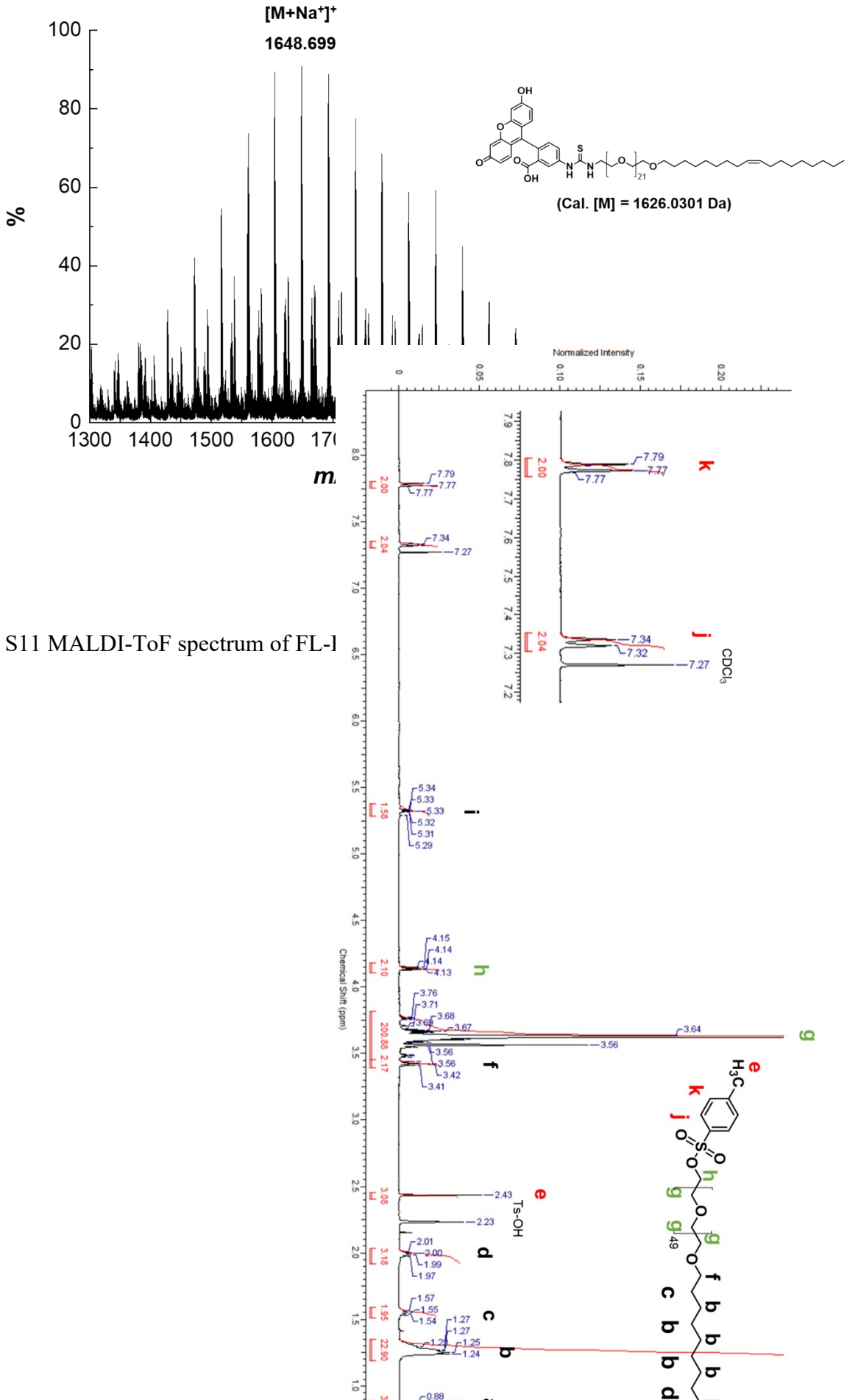


Fig. S11 MALDI-ToF spectrum of FL-l

Fig. S12 ^{1}H-NMR spectrum of Ts-

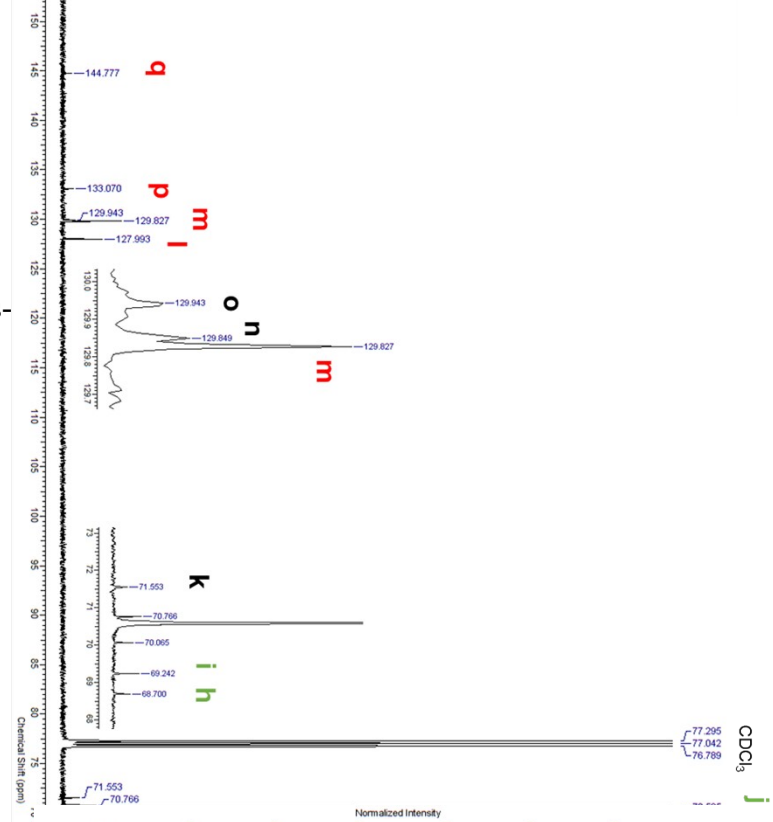


Fig. S13 ^{13}C-NMR spectrum of Ts-E

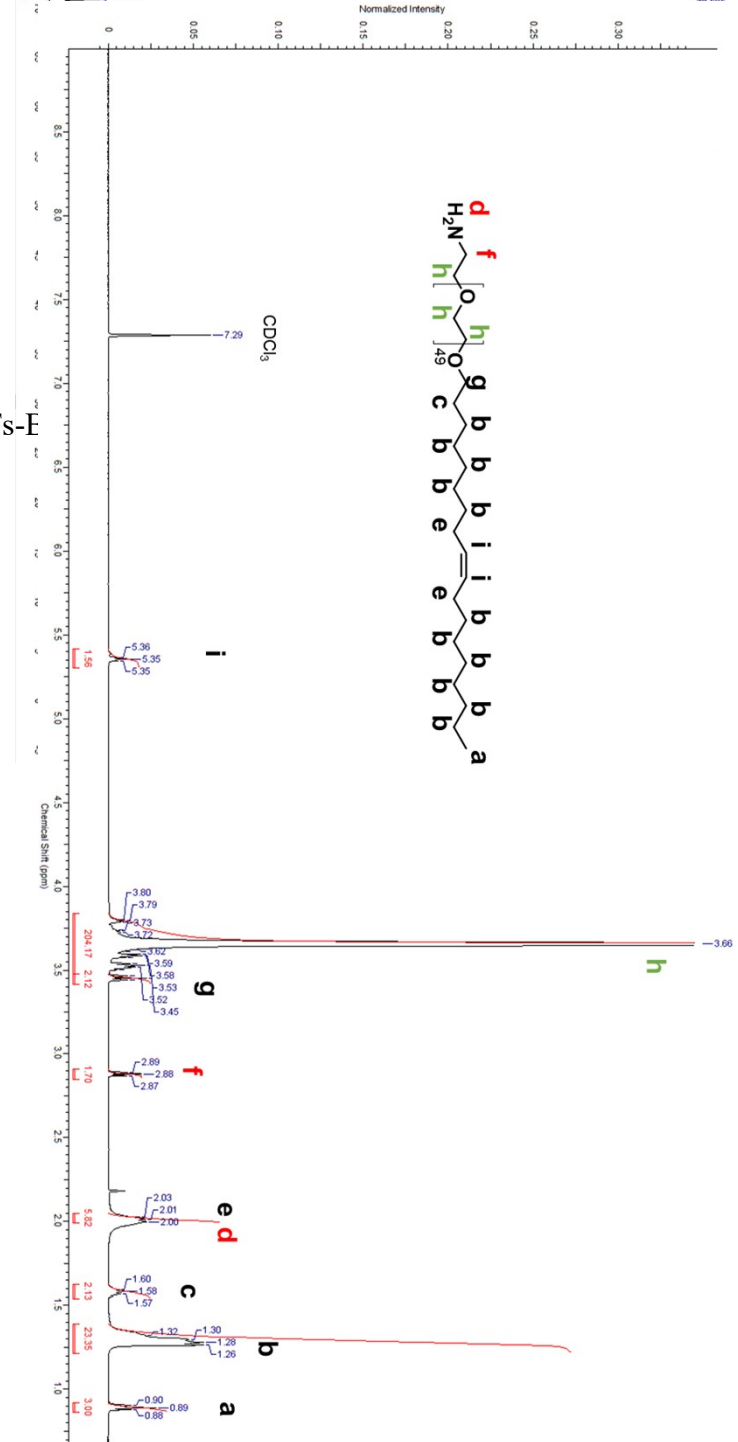


Fig. S14 ^{1}H-NMR spectrum of am

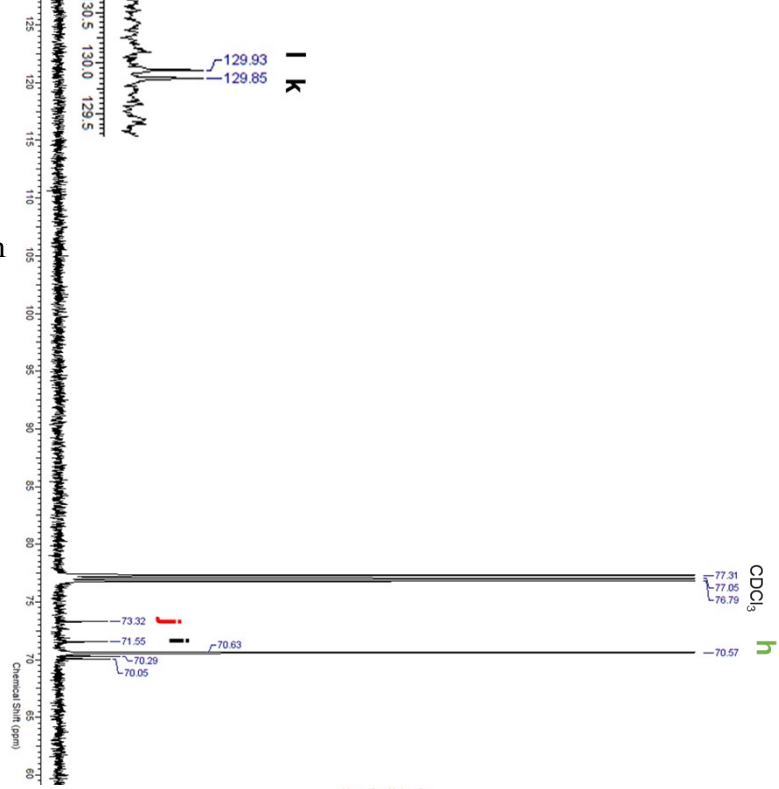


Fig. S15 ^{13}C-NMR spectrum of an

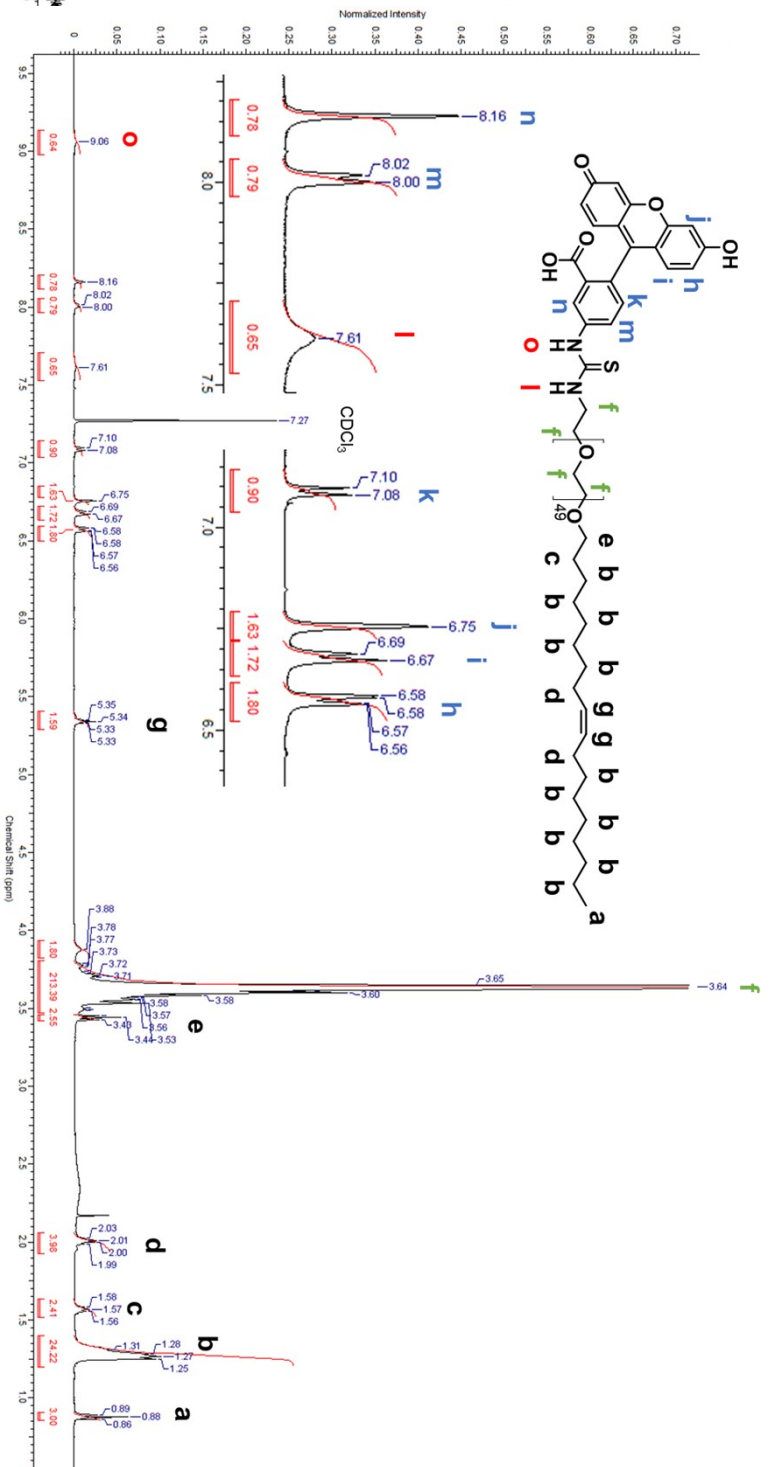


Fig. S16 ^{1}H-NMR spectrum of FL-

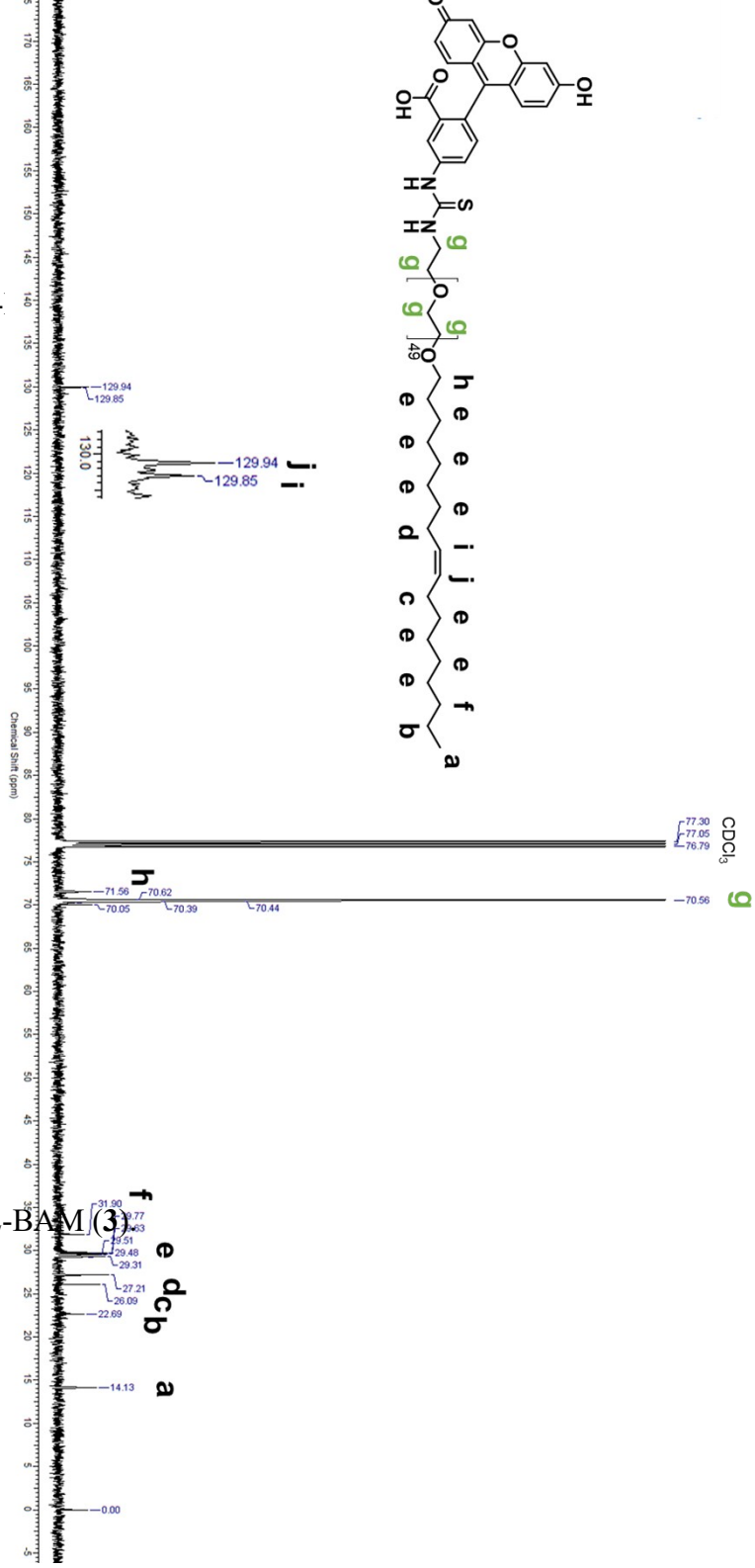


Fig. S17 ^{13}C-NMR spectrum of FL-BAM (3).

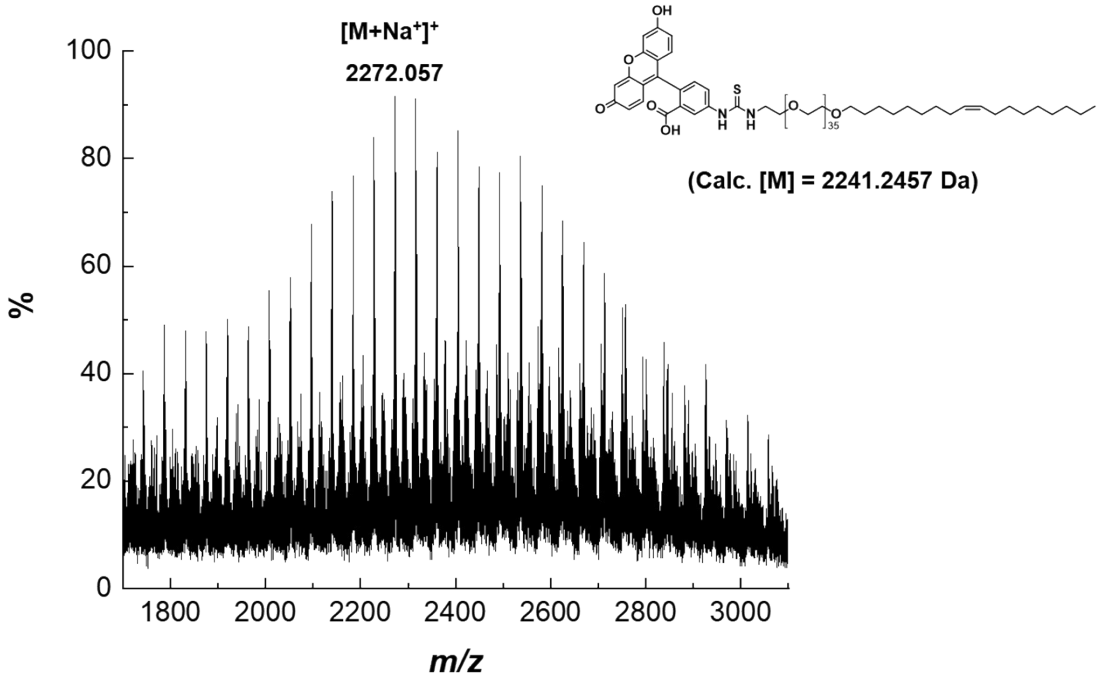


Fig. S18 MALDI-ToF spectrum of FL-BAM (**3**).

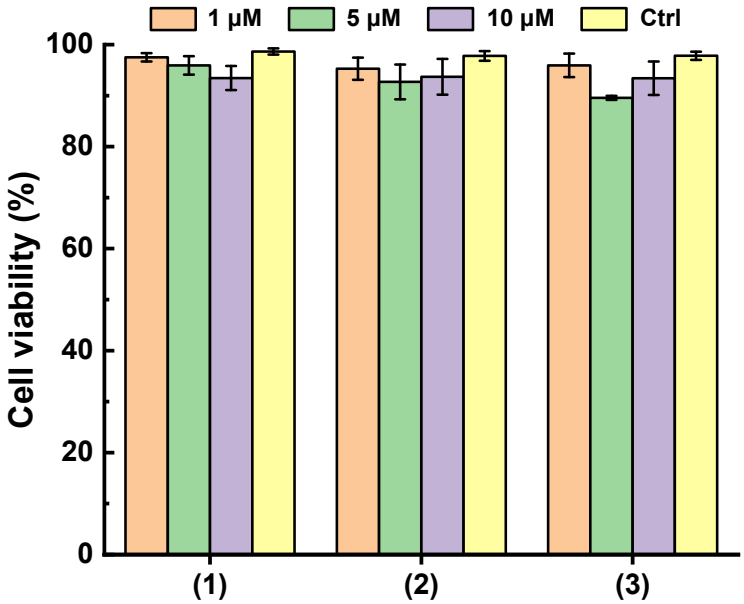


Fig. S19 Cell viability of NIH3T3 fibroblast cells treated with FL-BAM (**1**), FL-BAM (**2**), or FL-BAM (**3**), where control experiment was performed with intact cells.

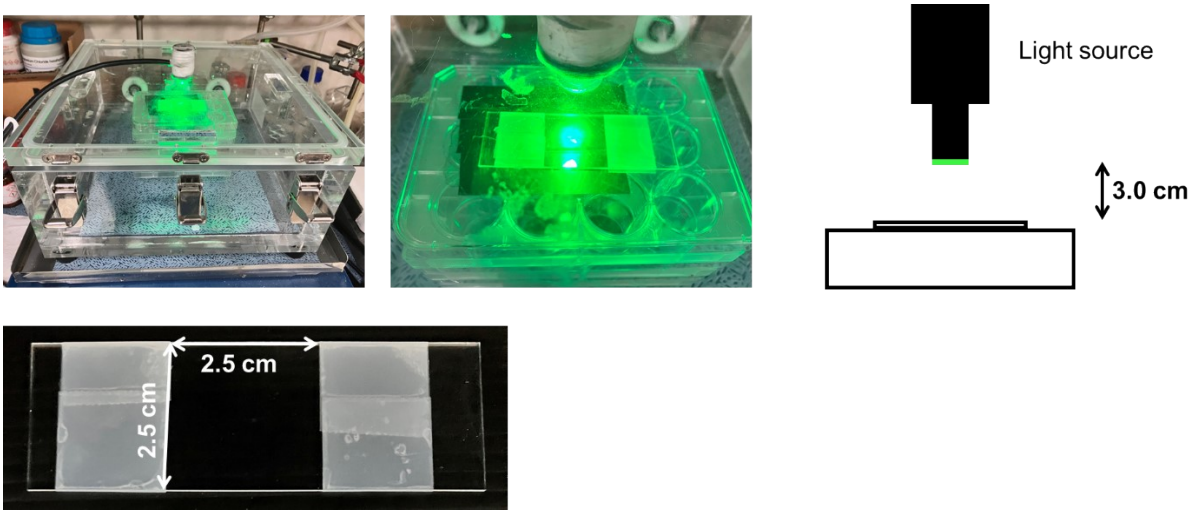


Fig. S20 Images of the photopolymerization setup.

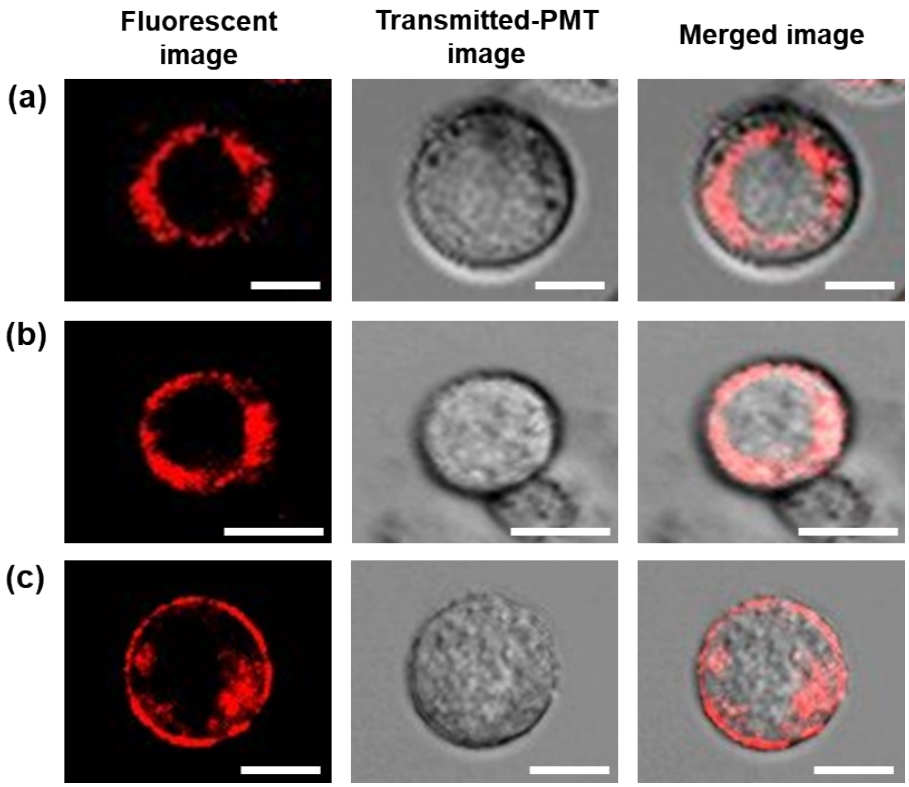


Fig. S21 Merged fluorescent and transmitted light images. (a) Cells treated with 5 μM of FL-BAM (**1**) for 2 min of incubation. (b) (a) cells further treated with TEOA (5 mM), VP (35 mM), and PEGDA (30 mM). (c) (b) cells then irradiated with visible LED light for 2 min. The scale bars indicated 10 μm.

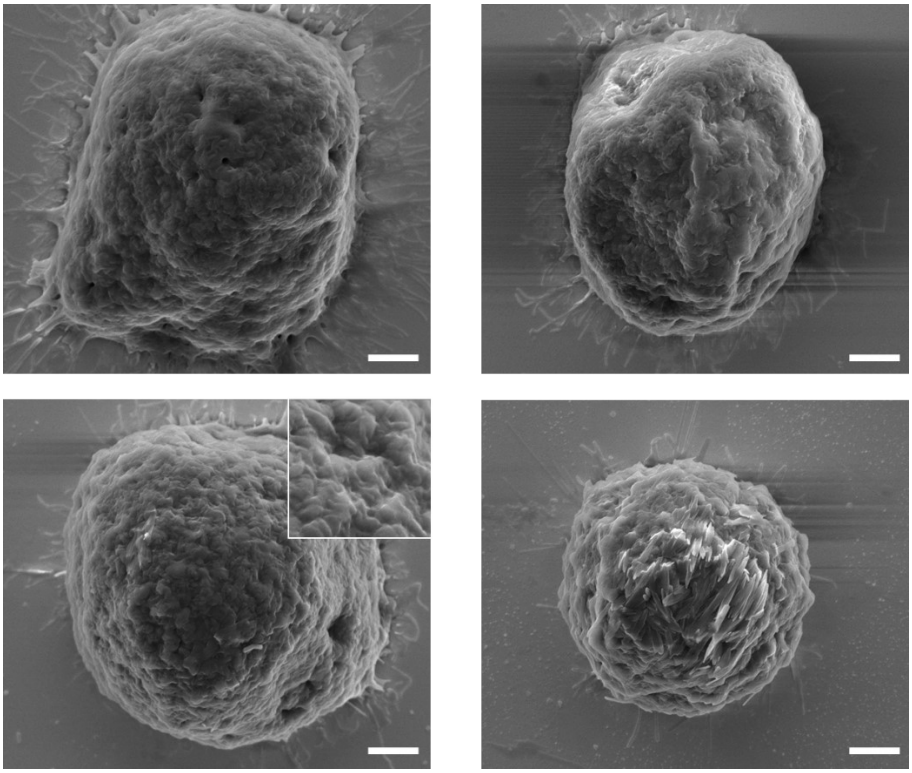


Fig. S22 SEM images of hydrogel coated NIH3T3 fibroblast cells. The scale bars indicate 2.5 μm.

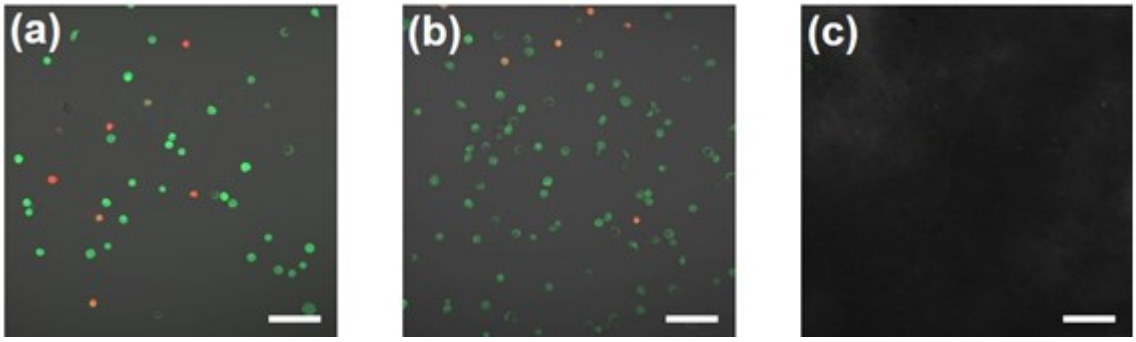


Fig. S23 CLSM images of encapsulated cells after (a) 1 day, (b) 2 days, and (c) 4 days. The gain value was set to 500 V during image acquisition. The scale bars represent 100 μm.

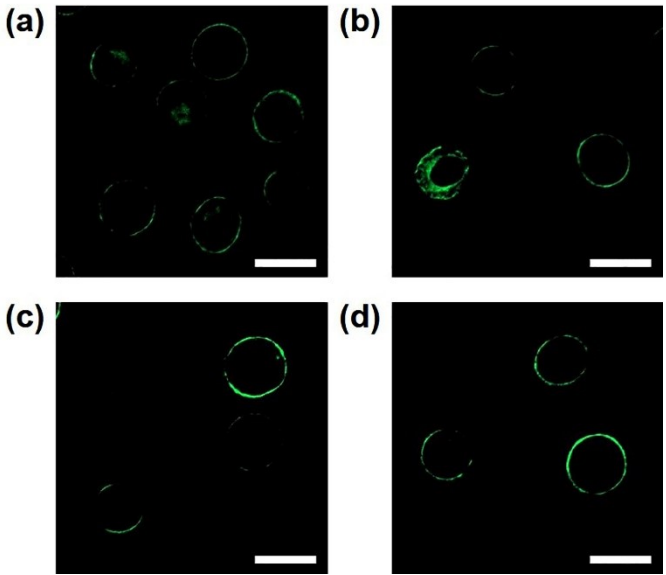


Fig. S24 CLMS images of NIH3T3 cells treated with FL-BAM (2) at concentrations of (a) 5 μM for 10 s, (b) 5 μM for 1 min, (c) 10 μM for 10 s, and (d) 10 μM for 1 min. The gain value was set to 500 V during image acquisition. The scale bars represent 20 μm.

Table S1. The effect of fluorescein concentration on the network structure of PEGDA hydrogels.

Fluorescein conc. (μM)	M_c (g/mol)[1]	Mesh size (Å)[1]
30	509	30
60	745	39
120	1295	60

5. Reference.

1. G. M. Cruise, D. S. Scharp and J. A. Hubbell, *Biomaterials,* 1998*,* **9***,* 1287.